REMARKS

The present invention concerns the indentification of targets of antibacterial agents using gene products from a bacteriophage that inhibits growth of the particular bacterium.

I. Modifications to the Drawings and the Specifications

New Figures 1A to 10 are submitted herewith to correct informalities noted by the Examiner.

As required by the Examiner, the section "Brief description of the Drawings" of the Specification has been amended to refer to all the frames shown in each of the figures.

As required also, the specification has been amended to remove embedded hyperlinks found on pages 59 and 60 of the application.

II. Response to Election/Restriction requirement

The Examiner has required restriction to one of the following invention under 35 U.S.C §121:

I. Claims 18-22, 25-27 and 28-29, drawn to a plurality of viral nucleic acids, to include vectors, host cells that comprise an open reading frame or an open reading frame fragment, classified in class 536, subclass 23.1.

II. Claims 23-24, 62-66, 107-110, drawn to proteins and peptides, classified in class 530, subclass 300.

III. Claims 1-6, 9-17, 32-38, 39-40, 77-106 drawn to a method of identifying antibacterial agent, classified in class 435, subclass 5.

IV. Claims 7-8, drawn to a method of identifying a mutated bacterial coding sequence, classified in class 536, subclass 24.32.

V. Claims 41-61, drawn to a method of inhibiting, treating or preventing infection, classified in class 424, subclass 204.1.

VI. Claims 67-70, drawn to a method of making an antibacterial agent, classified in class 514, subclass 2.

VII. Claims 71-76, drawn to an antibody and a method of using the antibody to detect a viral product, classified in class 435, subclass 7.1.

Applicants respectfully remind the Examiner that only claims 18, 23, 25, 28, 32 to 40 and 81 to 96 are pending since the other claims were cancelled in the amendment filed on May 4th 2001.

Applicants elect with traverse, claims 81 to 96 and new claims 111 and 112 for further prosecution (Group III).

The Applicants also submit that claims 23, 24 and new claims 113 to 118 should also be examined by the Examiner (Group II) in accordance with the discussion below.

The Office further requires that a single disclosed species be elected from the following species: open reading frame 12 and open reading frame 25. Applicants hereby elects open reading frame 25. All the pending claims (including new claims 111 to 118) are deemed to read on the elected species.

The Applicants respectfully traverse the restriction requirement on the ground that the Examiner has failed to follow MPEP guidelines for requiring restriction between Claim Groups II and III. Applicants respectfully remind the Examiner that the MPEP § 803 states as follows:

> "If the search and examination of an entire application can be made without a serious burden, the Examiner must examine it on the merits, even though it includes claims to distinct or independent inventions."

MPEP § 803 further states that "[t]here are two criteria for a proper requirement for restriction between patentably distinct inventions: (A) The inventions must be independent or distinct; and (B) There must be a serious burden on the examiner if restriction is required."

Applicants respectfully submit that a search of all the pending claims (Groups II and III) would not impose a serious burden on the Office. Indeed, because the elected claims of Group III require the use of the ORF 25 polypeptide, the Examiner will need to search for the product (ORF25 polypeptide) and this search will permit the Examiner to assess the patentability of the

claims directed to the ORF25 polypeptide (Claim Group II) without additional burden, and certainly without a "serious burden".

Therefore, in accordance with the direction of MPEP § 803, Applicants respectfully submit that the present restriction requirement is improper, and requests that Claim Group II be rejoined with Claim Group III for further prosecution in the present application.

III. Response to Examiner's General Observations

Examiner's General Observations

As indicated above, in connection with the inclusion of hyperlinks and/or other form of browser-executable code, Applicant has amended the cited portions of the Specification such that hyperlinks are no longer present, while retaining information directing one of ordinary skill in the art to the particular site.

In connection with the Examiner's inquiry concerning Tables 1-8, those tables were included in the Specification submitted to the Patent Office. That submission is evidenced by the receipt acknowledgment postcard returned by the Patent Office, on which the Tables were specifically noted. A copy of that postcard is attached for the Examiner's reference. If the Examiner is unable to locate Tables 1-8, Applicant can provide replacement copies as filed.

Applicant believes that the present application is now in condition for allowance. Favorable consideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

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APPENDIX 1 - MARKED UP VERSION OF AMENDED PARAGRAPHS SHOWING CHANGES MADE

FIGURES 1A, 1B and 1C [is a] are flow schematics showing the manipulations necessary to convert pT0021, an arsenite inducible vector containing the luciferase gene, into pTHA or pTM, two ars inducible vectors, or pTMLac, a lactose-inducible promot[o]er. [a)]Figure 1A: Vector pTHA contains BamH I and Sal I cloning sites and a downstream HA epitope tag. Figures 1B [b)] and [c)]1C: Vector pTM and pTMLac contain Bam HI and Hind III cloning sites and no HA epitope tag.

FIGURES 2A and 2B are schematic representations of the cloning steps involved to place the DNA segments of any of ORFs 12, 25, or other sequences into vectors to assess inhibitory potential. Figure 2A: [a)] For subcloning into pTHA, individual ORFs e.g. 44AHJD ORF 12 and 25 were amplified by the PCR using oligonucleotides targeting the start codon and the penultimated codon of the ORFs. Using this strategy, BamHI and SalI sites were positioned immediately upstream or downstream, respectively of these two codons. Following digestion with BamHI and SalI , the PCR fragments were subcloned into the same sites of pTHA.

Figure 2B: [b)] For subcloning into pTM or pTMLac, (examplified for pTM in b) individual ORFs were amplified by the PCR using oligonucleotides targeting the ATG and stop codons of the ORFs. Using this strategy, Bam HI and Hind III sites were positioned immediately upstream or downstream, respectively of the start and stop codons of each ORF. Following digestion with Bam HI and Hind III, the PCR fragments were subcloned into the same sites of pTM or pTMLac. Clones were verified by PCR and direct sequencing.

FIGURES 3A and 3B show schematic representations of the functional assays used to characterize the bactericidal and bacteriostatic potential of all predicted ORFs (>33 amino acids) encoded by bacteriophage 44AHJD. Fig. 3A:[)] Functional assay on semi-solid support media. Fig. 3B:[)] Functional assay in liquid culture.

FIGURES 4A and 4B show the results of the functional assay on semi-solid support media to identify bacteriophage 44AHJD ORFs with anti-microbial activity. Figure 4A [a)] shows the lists of the 31 bacteriophage 44AHJD ORFs that were screened in the functional assay and Figure 4B [b)] shows inhibition of bacterial growth following induction of expression of phage 44AHJD ORF 12 and 25 from three clones of *Staphylococcus aureus* transformants tested at four different concentrations. One clone of *Staphylococcus aureus* transformed with the non-inhibitory ORF (77 bacteriophage ORF 30 cloned into pT vector) was used as control. From these experiments, it is clear that expression of these two ORFs leads to the inhibition of growth of *Staphylococcus aureus*.

FIGURES [5 A] 5A and 5B are [the] graphs showing functional assays of [OD_{365} values and colony forming units (CFU) over time showing the results of functional assay in liquid mdia to assess] bacteriostatic or bactericidal activity of bacteriophage 44AHJD ORF 12 (Fig. 5A) and ORF 25 (Fig. 5B) in liquid media. The OD_{565} values (Frames 1 and 3) and colony forming units (CFU) over time (Frames 2 and 4) is shown. Growth inhibition assays were performed as detailed in the Detailed Description. The OD_{565} values and the number of CFU were determined from cultures of *Staphylococcus aureus* transformants harboring a given bacteriophage inhibitory ORF, in the absence or presence of the inducer. The identity of the expression vector and subcloned ORF harbored by the *Staphylococcus aureus* is given at the top of the each graph. The value of OD and the number of CFU was also determined from non-induced and induced control cultures of *Staphylococcus aureus* transformants harboring a non-inhibitory phage ORF cloned into the same vector. Each graph represents the average obtained from three *Staphylococcus aureus* transformants.

FIGURE 6 shows the pattern of protein expression of the inhibitory ORF in *S. aureus* in the presence or in the absence of induction with sodium arsenite. In individual inhibitory ORF (44AHJD phage ORF 12 and 25) cloned into the pTHA vector, the HA tag is set inframe with the

ORF and is positioned at the carboxy terminus of each ORF. An anti-HA tag antibody was used for the detection of the ORF expression. The identity of the subcloned ORF harbored by the *Staphylococcus aureus* transformants is given at the top of the panel.

FIGURES 7A and 7B depict the results from affinity chromatography using GST and GST/44AHJD ORF 25 as ligands with a *S. aureus* extract prepared by French pressure cell lysis and sonication. Eluates from affinity columns containing the GST and GST/ORF25 ligands at 0, 0.1, 0.5, 1.0, and 2.0 mg/ml resin were resolved by SDS-12.5% PAGE. Proteins were visualized by silver staining. Micro-columns were eluted with: A) 1 M NaCl ABC (ACB; 20 mM Hepes pH 7.5, 10 % glycerol, 1 mM DTT, and 1 mM EDTA); and B) 1% SDS. Each molecular weight marker is approximately 100 ng. The lanes labeled ACB indicate eluates from a 2.0 mg/ml ligand column loaded only with ACB buffer containing 75 mM NaCl. The arrows indicate proteins specifically with GST/ORF25.

FIGURE 8 shows results of a tryptic peptide mass spectrum of the PT48 protein that interacted with 44AHJD ORF 25 and that was eluted with 1% SDS and labelled: PT48 in Figure 7B. The control band excised from the 48 kDA region of the gels [containing PT48] did not contain PT48.

FIGURE 9 shows the identification of PT48 as *S. aureus* DNA-directed DNA polymerase III beta subunit protein from the Genbank™ database (accession number: [1084187] 1084187).

FIGURES 10A and 10B show the nucleotide sequence (Fig. 10A; SEQ ID NO: 166) and amino acid sequence[s] (Fig. 10B; SEQ ID NO: 167) of *S. aureus* DnaN.

Paragraph at p.59, line 23 to p.60, line 8:

Sequence homology (BLAST) searches for each ORF are then carried out using an implementation of BLAST programs, although any of a variety of different sequence comparison and matching programs can be utilized as known to those skilled in the art. Downloaded public databases used for sequence analysis include:

i) non-redundant GenBank (ftp site with the remainder of the address being [://]ncbi.nlm.nih.gov/blast/db/nr.Z),

ii) Swissprot (ftp site with the remainder of the address being [://]ncbi.nlm.nih.gov/blast/db/swissprot.Z);

iii) vector (ftp site with the remainder of the address being [://]ncbi.nlm.nih.gov/blast/db/vector.Z);

iv) pdbaa databases (ftp site with the remainder of the address being [://] ncbi.nlm.nih.gov/blast/db/pdbaa.Z);

v) *Staphylococcus aureus* NCTC 8325 (ftp site with the remainder of the address being [://]ftp.genome.ou.edu/pub/staph/staph-1k.fa);

vi) *Streptococcus pyogenes* [streptococcus pyogenes] (ftp site with the remainder of the address being [://]ftp.genome.ou.edu/pub/strep/strep-1k.fa);

vii) *S*[*s*]*treptococcus pneumoniae* (ftp site with the remainder of the address being [://]ftp.tigr.org/pub/data/s_pneumoniae/gsp.contigs.112197.Z);

viii) *M*[*m*]*ycobacterium tuberculosis* CSU#9 (ftp site with the remainder of the ix) address being [://]ftp.tigr.org/pub/data/m_tuberculosis/TB_091097.Z) and

ix) *P*[*p*]seudomonas *aeruginosa* ([http://www.] world wide web site with the remainder of the address being genome.washington.edu/pseudo/data.html).

APPENDIX 2 - MARKED UP VERSION OF AMENDED CLAIMS SHOWING CHANGES MADE

23. (Amended) An isolated, purified, or enriched polypeptide comprising at least a fragment of a protein encoded by *Staphylococcus aurous* bacteriophage 44AHJD open reading frame ~~12 or~~ 25, wherein said portion is at least 5 amino acid residues in length.

24. (Amended) The polypeptide of claim ~~24~~ 23, wherein said polypeptide comprises a fragment at least 10 amino acid residues in length of a said polypeptide normally encoded by said bacteriophage.

81. (Amended) A method of screening for compounds that inhibit an *S. aureus* dnaN product, comprising

contacting together: i) a bacteriophage 44AHJD ORF25 product or a functional fragment thereof; ii) a dnaN product having an interaction with said 44AHJD ORF25 product or said ORF25 functional fragment; and iii) ~~said dnaN product with a bacteriophage 44AHJD ORF25 product or a fragment thereof and~~ at least one test compound; ~~,~~ and

determining whether ~~any of~~ said at least one test compounds reduces the interaction between said dnaN product and said ORF25 product or functional fragment, wherein a reduction in said interaction is indicative that said test compound inhibits said *S. aureus* dnaN product.

83. (Amended) The method of claim 81, wherein said determining comprises measuring the interaction between said dnaN product and said ORF 25 product or functional fragment, wherein said dnaN or ORF25 product or functional fragment is directly labeled.

84. (Amended) The method of claim 83, wherein said dnaN product comprises an active portion, a mimetic, a corresponding isolated, enriched, or purified protein, or a homologous product of a *S. aureus* dnaN gene.

85. (Amended) The method of claim ~~83~~ 81, wherein said dnaN or ORF25 product is indirectly labeled.

86. (Amended) The method of claim 81, wherein said determining ~~detecting~~ comprises measurement by phage display.

87. (Amended) The method of claim 81, wherein said determining ~~detecting~~ comprises measurement by surface plasmon resonance.

88. (Amended) The method of claim 81, wherein said determining ~~detecting~~ comprises measurement by Fluorescence Resonance Energy Transfer.

89. (Amended) The method of claim 81, wherein said determining ~~detecting~~ comprises measurement of fluorescence polarization changes.

90. (Amended) The method of claim 81, wherein said determining ~~detecting~~ comprises a scintillation proximity assay.

91. (Amended) The method of claim 81, wherein said determining ~~detecting~~ comprises a biosensor assay.